84-2 Unscheduled DNA Synthesis in Cultured Human Fibroblasts
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DATA EVALUATION REPORT

Study Type: DNA Repair Assay in Cultured Human Fibroblasts
TOX. CHEM. NO.: 298C
Accession No.: 403888-11
MRID NO.:

Test Material: CGA-154281 Technical (Batch No. KGL 3339-6; 99.7% Purity)

Synonyms:

Study Number (s): 850666

Sponsor: CIBA-GEIGY Corp.

Testing Facility: Experimental Pathology, CIBA-GEIGY Limited, Basle, Switzerland

Title of Report: Autoradiographic DNA Repair Test on Human Fibroblasts

Author(s): E. Puri, I. Hunziker and K. Mennle

Report Issued: October 15, 1985

Conclusions:

CGA-154281 Technical did not cause DNA damage or inducible repair in human fibroblast unscheduled DNA synthesis without metabolic activation at the concentrations tested.

Concentrations tested: 0.25, 1.25, 6.25 and 31.25 ug/ml

Classification of Data: Unacceptable

(Deficiencies are identified in the detailed review)

Title of Study: Autoradiographic DNA-Repair Test on Human Fibroblasts
(Test Material: CGA-154281 Technical)
Ciba-Geigy Limited Experimental Pathology Laboratory
Test No. 850666

I. Materials and Methods:

1. Test Materials

The test compound, CGA-154281 Technical (Batch No. KGL 3339-6; 99.7% Purity), dissolved in DMSO was used in this study. 4 - nitroquinoline-N-oxide (5 uM) was used as the positive control.

2. Medium

Dulbecco's minimal essential medium containing 10% fetal bovine serum.

3. Indicator Cells

The cell line of Human Fibroblasts obtained from the American Type Culture Collection (CRL 1121) was used in this study.

4. Cell Preparation

A series of compartments in multiplates containing glass coverslips were seeded with 3×10^4 cells per compartment (1 ml medium/compartment) and cultivated overnight. Monolayer cultures were established on coverslips in culture plates for initiation of the UDS assay.

5. Preliminary Cytotoxicity Test

Attached human fibroblasts were exposed to seven concentrations of CGA-154281 technical (31.25 through 2000 ug/ml) for 5 hours. After exposure, cells were washed with BSS and stained with Trypan-Blue solution (0.2%) for 5 minutes. Again, after washing with BSS, the Cells were fixed and the percentage of unstained cells evaluated by counting 100 cells.

6. UDS Assay

The human fibroblasts attached on coverslips (3×10^4 viable cells) were used in this assay. Following the addition of CGA-154281 technical (0.25, 1.25, 6.25 and 31.25 ug/ml) and ^{3}H-thymidine (2 uCi/ml) in each compartment with 1 ml of medium, the culture compartments were incubated for 5 hours. After incubation, the treated cultures were washed with BSS and fixed with ethanol/acetic acid (3:1). The coverslips were mounted on microscope slides and prepared for autoradiography. The exposure time was 6 days. The autoradiographs were stained with hematoxylin-eosine.

7. Grains Counting

From each of the treatment groups and from the positive and the negative controls, 150 nuclei in altogether three slides were scored. Counting of silver grains over the nuclei of the fibroblasts was carried out with the aid of an electronic counter (ARTEK Model 982) attached to microscope at a magnification of 2000 X.

8. Evaluation Criteria

The test material is generally considered to be mutagenic if the mean number of silver grains per nucleus in relation to the negative control is more than doubled at any concentration.

II. Reported Results:

1. Preliminary Cytotoxicity Test

From the results obtained in the cytotoxicity test, the highest usable concentration was found to be 31.25 ug/ml. Three lower concentrations (diminishing by a factor of 0.2) were also selected for this study.

2. UDS Assay (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 attached)

Comparison of the mean number of silver grains per nucleus in the negative controls and after treatment with CGA-154281 Technical in the various concentrations (0.25, 1.25, 6.25 and 31.25 ug/ml) revealed no marked differences. By contrast, the positive control, 4-NQO (5 uM) yielded a marked increase in the mean value of silver grains per nucleus when compared to that of the negative controls (4-NQO, 31.6; Medium control, 1.30; DMSO control, 0.87).

III. Evaluation and Recommendation:

The evaluation of the DNA-damaging effects of CGA-154281 Technical in the unscheduled DNA synthesis in cultured human fibroblasts cannot be accomplished due to the following reporting deficiencies:

1. The results obtained from the preliminary cytotoxicity test for dose selection were not given in this report. Sufficient data should be provided to support the author's conclusion.

2. The protocol used for the autoradiography was not presented. For autoradiographic determinations, the value of ^{3}H-thymidine incorporated in the cytoplasm should be substracted from the number of grains found over the cell nucleus to give the net incorporation

84-2 - Unscheduled DNA Synthesis in Rat Hepatocytes

Reviewed by: John H.S. Chen 2/16/88
Section I, Toxicology Branch (TS-769C)
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Section VI, Toxicology Branch (TS-769C)
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Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: DNA Repair Assay in Rat Hepatocytes **TOX. CHEM. NO.:** 2980

Accession No.: 403888-10 **MRID NO.:**

Test Material: CGA-154281 Technical (Batch No. KGL 3339-6; 99.7% Purity)

Synonyms:

Study Number (s): 850665

Sponsor: CIBA-GEIGY Corp.

Testing Facility: Experimental Pathology, CIBA-GEIGY Limited, Basle, Switzerland

Title of Report: Autoradiographic DNA Repair Test on Rat Hepatocytes

Author(s): E. Puri, I. Hunziker, and K. Mennle

Report Issued: October 21, 1985

Conclusions:

CGA-154281 Technical did not cause DNA damage or inducible repair in rat hepatocyte unscheduled DNA synthesis at the concentrations tested (1 through 125 ug/ml).

Concentrations tested: 1, 5, 25 and 125 ug/ml

Classification of Data: Unacceptable

(Deficiencies are identified in the detailed review)

Title of Study: Autoradiographic DNA Repair Test on Rat Hepatocytes
(Test Material: CGA-154281 Technical)
Ciba-Geigy Limited Experimental Pathology Laboratory
Test No. 850665

I. Materials and Methods:

1. Test Materials

The test compound, CGA-154281 Technical (Batch No. KGL 3339-6; 99.7% Purity), dissolved in DMSO was used in this study. Dimethylnitrosamine (100 mM) was used as the positive control.

2. Medium

Williams' medium E containing 10% fetal bovine serum

3. Indicator Cells

The hepatocyte obtained from a male rat (TIF. RAIF(SPF); 340 g) was used in this study.

4. Cell Preparation

Recovered cells were seeded at 4 X 10^5 cells in a series of compartments in multiplates containing gelatinized THERMANOX coverslips. The cells were allowed to attach to the cover-slip during an attachment period of 1.5 - 2 hours. After incubation, cells were washed and cultivated overnight in fresh medium.

5. Preliminary Cytotoxicity Assay

Attached primary rat liver cells were exposed to seven concentrations of CGA-154281 Technical (31.25 through 2000 ug/ml) for 5 hours. After exposure, cells were washed with BSS and stained with Trypan-blue solution (0.2%) for 5 minutes. After washing with BSS again, the cells were fixed and the percentage of unstained cells evaluated by counting 100 cells.

6. UDS Assay

The freshly isolated rat liver cells attached on coverslips (4 X 10^5 viable cells) were used in this assay. Following the addition of CGA-154281 Technical (1, 5, 25 and 125 ug/ml) and ^{3}H-thymidine (8 uCi/ml) in the culture dishes with 2 ml of medium, the culture dishes were incubated for 5 hours. After incubation, the treated cultures were washed with BSS and fixed with ethanol/acetic acid (3:1). The cover slips were mounted on microscope slides and prepared for autoradiography. The exposure time was 6 days. The autoradiographs were stained with hematoxylin-eosine.

7. Grain Counting

From each of the treatment groups and from the positive and the negative controls, 150 nuclei in altogether three slides were scored. Counting of silver grains over the nuclei of the hepatocytes was carried out with the aid of electronic counter (ARTEK Model 982) attached to a microscope at a magnification of 2000 X.

8. Evaluation Criteria

The test material is generally considered to be mutagenic if the mean number of silver grains per nucleus in relation to the negative controls is more than doubled at any concentration.

II. Reported Results:

1. Preliminary Cytotoxicity Test

From the results obtained in the toxicity test, the highest usable concentration was found to be 125 ug/ml. Three lower concentrations (diminishing by a factor of 0.2) were also selected for this study.

2. UDS Assay (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 attached)

Comparison of the mean number of silver grains per nucleus in the negative controls and after treatment with CGA-154281 in the selected concentrations revealed no marked increase after treatment with CGA-154281. The highest concentration of the test material (125 ug/ml) reduced the number of silver grains to below the vehicle control value (factor: 0.41). By contrast, the positive control, DMN (100 mM) yielded a marked increase in the mean value of silver grains per nucleus when compared to that of the negative controls (DMN, 18.6; DMSO, 3.15).

III. Evaluation and Recommendation:

The evaluation of the DNA-damaging effects of CGA-154281 Technical in the unscheduled DNA synthesis in rat hepatocytes cannot be accomplished due to the following reporting deficiencies:

1. The detailed procedure used for the isolation of adult rat hepatocytes (Perfusion Technique) and the complete components of Williams' medium E used in this assay were missing.

2. The results obtained from the preliminary cytotoxicity test for dose selection were not given in the report. Sufficient data should be provided to support the author's conclusion.

3. The protocol used for the autoradiography was not presented. For autoradiographic determinations, the value of ^{3}H-thymidine incorporated in the cytoplasm should be substracted from the number of grains found over the cell nucleus to give the net incorporation rate which should be reported as net grains per nucleus in this study (Reference: William, G.M.

Cancer Res., 37: 1845-1851, 1977).

4. According to the acceptable criteria for determining a positive response in the UDS assay recommended by William (Cancer Res., 37: 1845-1851, 1977), a test material must induce a significant increase in net grain count per nucleus (not in silver grain count per nucleus) in relation to the vehicle control. Therefore, the evaluation criteria established by the testing laboratory for this study appears to be inappropriate and must be clarified. The results of this study should be calculated in terms of the increase in net grain count per nucleus at the applied concentrations.

5. Test not repeated to confirm.

6. Since the submitted report is inconclusive, the study is unacceptable in the present form. However, it may by upgraded on resolution of the reporting deficiencies.

4-(Dichloroacetyl)-3,4-Dihydro-3-Methyl-
2H-1,4-Benzoxazine

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